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Molecular Profiling of *Escherichia coli* O157:H7 and Non-O157 Strains Isolated from Humans and Cattle in Alberta, Canada

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Virulence markers in Shiga toxin-producing *Escherichia coli* (STEC) and their association with diseases remain largely unknown. This study determines the importance of 44 genetic markers for STEC (O157 and non-O157) from human clinical cases and their correlation to disease outcome. STEC isolated from a cattle surveillance program were also included. The virulence genes tested were present in almost all O157:H7 isolates but highly variable in non-O157 STEC isolates. Patient age was a significant determinant of clinical outcome.

Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157 strains have been identified in outbreak settings associated with water (1), food (2, 3, 4, 5), and animals (6, 7). Shiga toxin (Stx) production, especially Stx2 and subtypes Stx2a, Stx2c, and Stx2d (8, 9, 10, 11), has been implicated in causing severe disease and hemolytic uremic syndrome (HUS) (8, 12, 13, 14, 15, 16, 17, 18). Since not all patients infected with Stx2-producing STEC develop HUS, additional virulence factors (8, 17, 19) are likely required for disease. This study establishes the molecular profiles for a panel of selected clinical O157:H7 and non-O157 STEC isolates from humans and cattle surveillance in Alberta, Canada. The clinical outcome was correlated to molecular profiles to find genetic determinants of virulence and HUS.

Pulsed-field gel electrophoresis (PFGE) data from 1,407 human clinical O157:H7 isolates from 2004 to 2012 were analyzed, and a subset of 89 strains was selected from outbreak and sporadic settings. They were chosen to maximize the time period and the diversity of PFGE fingerprint patterns represented. *E. coli* O157 isolates from all HUS cases and clinical non-O157 STEC ($n = 39$) from the Provincial Laboratory for Public Health (ProvLab) and Alberta Agriculture and Rural Development (AARD) cattle surveillance ($n = 19$) collections were included. DNA from these isolates was extracted using the MagaZorb DNA mini-prep kit (Promega Corporation, Madison, WI, USA). The 44 genetic markers selected (Table 1) were investigated by high-throughput microfluidic real-time PCR amplification (20). The genes stx_1 and stx_2 were detected using real-time PCR (21), followed by subtyping (22). The serotypes of non-O157 STEC isolates were determined using conventional methods. χ^2 values, *P* values, and statistical comparison of means using unpaired two-tailed *t* tests were calculated using the GraphPad QuickCalcs website (GraphPad Software, La Jolla, CA). Relative risks (RR), 95% confidence intervals (CI), and corresponding *P* values were calculated using MedCalc (version 12.7.7; MedCalc Software, Ostend, Belgium). A *P* value of <0.05 was considered to be statistically significant. Patient demographic data and disease outcome were provided by Alberta Health.

O26:H11 or O26:HNM ($n = 8$; 20.5%) was the most common serotype observed in human isolates in this study, followed by O111:HNM, O111:HNT, or O111:H8 ($n = 6$; 15.4%) and O121:H19 ($n = 5$; 12.8%). O109:H5 or HNM ($n = 3$; 15.8%) was most common in cattle isolates followed by O26:H11, O84:HNM, and O98:HNM ($n = 2$; 10.5% each). The virulence genes tested were present in 96.6% to 100.0% of the O157:H7 strains, with the exception of stx_1, which was only positive in 75.3% ($n = 67$) of the isolates (Table 2). stx_1 (79.5% to 84.2%) was more prevalent than stx_2 (35.9% to 36.8%) in the non-O157 isolates. The following genes were present in >80% of the human clinical non-O157 strains: *eae*, *ehxA*, *ent*, *nleB*, *nleE*, *efa1*, *nleF*, *nleH1-2*, *ureD*, *terE*, *espK*, *espM2*, *espW*, *espG*, *espF1*, *espX1*, *espR1*, and *espJ* (Table 2), compared to only stx_1 and *espR1* in cattle strains. Also, genes *eae*, *toxB*, *katP*, *ent*, *nleB*, *nleE*, *efa1*, *efa2*, *nleF*, *nleH1-2*, *nleA*, *ureD*, *terE*, *espV*, *espK*, *ecs1763*, *espM1*, *espN*, *espM2*, *espX7*, *espW*, *espG*, *espF1*, *espX1*, *nleH1-1*, *nleG*, *espJ*, and *ecs1822* were significantly more prevalent in non-O157 human strains (Table 2).

The O157 and non-O157 isolates were categorized into seropathotypes A, B, and D/E (Table 2). Seropathotype C was excluded from the analysis because it contained only two isolates. Additionally, five isolates could not be grouped into a seropathotype. The gene distributions of isolates in seropathotype A and O157:H7 categories were identical. The virulence genes tested were most prevalent in seropathotype A, followed by seropathotypes B and then D and E. Several genes from CP-933N (*espK*), LEE (*eae*, *espG*), OI-44 (*espV*), OI-50 (*espN* and *espX7*), OI-57 (*ecs1763*), OI-71 (*ecs1822*, *espM1*, *nleF*, and *nleG*), OI-79 (*espJ*), OI-108 (*espM2* and *espW*), and OI-122 (*efa1*, *efa2*, *ent*, *nleB*, and

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TABLE 1 Virulence gene targets and oligonucleotide sequences used in the quantitative PCR microarray

Virulence gene targets	Forward primer/reverse primer
eae	CATTGATCAGGATTTTTCTGGTGATA/CTCATGCGGAAATAGCCGTTA
eae-gamma	GACTGTTAGTGCGACAGTCAGTGA/TTGTTGTCAATTTTCAGTTCATCAAA
ecs1763	TACTCTTGACCTTAGTACCGC/TTTTGATTATCAGAACCATATAGCCC
ecs1822	CCGTACAGCGTGATTCATCC/TAGCGAAACGGGCAAGGTC
efa1	TTTTCACCAGTTCATCATACAGG/CCATTATAAACATTTGCCAGACC
efa2	ACTAAGATCAATACAAGGATTCC/ATCCATCAGGCCATAGGTG
ehxA	CGTTAAGGAACAGGAGGTGTCAGTA/ATCATGTTTTCCGCCAATGAG
ent	TCCTGGATTATTTTCTGCATTTCA/ACTATTGCCAAGTACGCCACAA
espF1	TCGYCCGGCMCCRCCG/TGCCTGWGCAATGGGCGG
espG	AGCTGAAGTTGTGGARTTTTTATGC/TGTGTCAACRTTAAGGCTGGCA
espJ	AAAGGAGCMAAAGTATATCCMGATA/AACATCSASYCTRACTGWTTCTGG
espK	GCAGRCATCAAAAGCGAAATCACACC/TCGTTTGGTAACTGTGGCAGATACTCG
espM1	TCAGCTCTTTTGGTATCA/CGCTAAATTTGTTAACATTAAAG
espM2	CAGCACAAAGTTCTATTAATCATGTRATAATGGGG/ACTTGTCCGCAAGCAAGTTTGCTATG
espN	GACATATTTGTTTATGTCATCAGGAGCGG/CCTCAGGATATGGATGGCCTACTGGC
espO1-1	CATGTTGTTGATGTAAGTATGCAG/AAGTTCACAAGTACATTACCCGG
espP	ATGCCCCGTCAGCATCTG/TCGACCGTCAGCGTATGG
espR1	GCTCATGTTATTTAATTTRTTTTCCC/AGTGAAACAATTCGTTATCCACATC
espV	TCAGGTTCCTCGTCTGATGCCGC/CTGGTTCAGGCCTGGAGCAGTCC
espW	CCAAACTTAGGAGAGCGAGACGTAAGAATG/CCTGAATAAAGATACTCACCTAACTCTG
espX1	ACTCAACTTGTCAATAAGCACTTAGG/ACACATCATTATTTAGCTTTACATTGTC
espX2	TGTTATCATCCGATAACTCTGGG/GTCTTTTTCTTAACCTCTGGCTC
espX6	TCACAACCGTGATTCTATATGAAAC/TTAGTATTGCGCCAGTAAGAATTAC
espX7	TGCTGAAGAATTGAATTACTCT/TGTATCATCAAGCACTGCTCC
espY1	ACTTCTGTTCAATTTCGTGAATAAAA/ATTCCAGACTATGGATAAACTTTTCTT
espY3	GAACTATCTCTATTGTTAACGAGG/CTAGATATAGACCGCTGAAATCG
espY4-2	GCAATGTATAAGGAGAGGCTTAGTC/TTGACTTCTTTAATATGAGAGCCAGG
etpD	TTGGATGACGGCGAAACTG/AGATGATACGCTGTTGGGAG
iha	AGTGGTACGGGTAAAACCG/AGTATCAGCGTGTAACTGGC
katP	GAAGTCATATATCGCCGGTTGAA/GTCATTTCAGGAACGGTGAGATC
lpfA	TACTGTCCGTTGACTCTCAG/ACCAACCGCAGCAAATACAG
nleA	AGATAACYCTAATACTAAATATGCC/GCCCAACCATTGCRCCGATATGAGG
nleB	CATGTTGAAGGCTGGAASTTTGT/CCGCTACAGGGCGATATGTT
nleD-2	GGACTGGTTCCGATTTTCACTG/AAAGTCCACCACGCTAATCCC
nleE	AGAAGCGTTTGAACCTATTTCCA/TTGGGCGTTTTCCGGATAT
nleF	TGAGGTGAGAAATGAAAATACTGATG/CTATCCCTGTCCTCTATCGTCATTC
nleG	CCAGATTTTCTGCGAGAAGGC/GGACAGTTTTAGCGTGGAACC
nleH1-1	TACCGAGTGTGGACTATA/CAGGACTTTTGTTGCATC
nleH1-2	ACAAGAGAAAGTCATAGTGGTTG/AATCTCYCCCTTAGGCCATCCCA
pagC	GGCTGATAATCATACGCTATCG/ATCGATATTGCAGATTCACTCC
stx, all variants	TTTGTYACTGTSACAGCWGAAGCYTTACG/CCCCAGTTCARWGTRAGRTCMACRTC
terE	GCCGTTACCATCTATGATGC/TGTAAACGCGCATGAAGCTG
toxB	AGTATCAGTCACATAAAGTAGAC/GCATTRGGATCAATCCAGAG
ureD	GCAATAATTGACTCTGATTGCC/GCTGCTGCGGTAAAATTTACT

nleE) were significantly more prevalent in seropathotypes A and B than in D and E.

A total of 67 (75.3%) O157:H7 isolates were positive for both stx_{1a} and stx_{2a}, 19 (21.3%) were positive for stx_{2a} only, and 3 (3.4%) were positive for both stx_{2a} and stx_{2c} (Table 3). The stx_1 and stx_2 genes were present in 31 (79.5%) and 14 (35.9%) of the non-O157 strains isolated from humans, respectively (Table 3). Subtypes stx_{1c} and stx_{2c} were detected in this category, but the majority of them were stx_{1a} only ($n = 22$; 56.4%) (Table 3). In the cattle isolates, similar results were obtained ($n = 12$; 63.2%) (Table 3).

None of the non-O157-infected patients reported HUS; of the 1,407 O157:H7-infected patients, 29 developed HUS and required hemodialysis, but no deaths or kidney transplants were reported. The mean age of HUS patients was 8.7 years and was significantly lower than that of non-HUS patients infected with O157:H7 (mean age, 30.3 years; $P < 0.0001$) and non-O157-infected patients (mean age, 22.0 years; $P = 0.0057$) (Table 4). Of the HUS patients, 23 (79.3%) were <10 years of age, 14 of which were <5 years of age (Table 4). In comparison, only 440 of the 1,407 (31.3%) total O157:H7-infected cases during the study period were <10 years of age. Non-O157-infected patients of all age categories were observed, with the most common age category being <5 years ($n = 12$; 30.8%). No significant gender differences were observed for the O157:H7-infected patients (relative risk [RR], 1.05; 95% confidence interval [CI], 0.97 to 1.13; $P = 0.21$), O157:H7-infected patients with HUS (RR, 1.41, 95% CI, 0.83 to 2.41, $P = 0.20$), or non-O157-infected patients (RR, 0.77; 95% CI, 0.49 to 1.21; $P = 0.26$). While all HUS patients were hospitalized, only 345 (24.5%) of the 1,407 O157:H7-infected and 3 (7.7%) non-O157-infected patients required hospitalization (Table 4).

TABLE 2 Frequency and distribution of virulence genes in *E. coli* O157:H7 and non-O157 and seropathotypes

Genetic location[a]	Virulence gene	Gene presence (no. [%]) in:			Seropathotype (no. [%])			
		O157:H7 ($n = 89$)	Non-O157 from human ($n = 39$)	Non-O157 from cattle ($n = 19$)	B ($n = 29$)	D/E ($n = 22$)	χ^{2b}	*P* value
Stx phage	stx_1	67 (75.3)	31 (79.5)	16 (84.2)	23 (79.3)	19 (86.4)	0.005	0.9411
Stx phage	stx_2	89 (100)	14 (35.9)	7 (36.8)	10 (34.5)	8 (36.4)	0.005	0.9440
C-I	*espY1*	89 (100)	2 (5.1)	0 (0)	2 (6.9)	0 (0.0)	0.057	0.8119
C-I	*espY3*	89 (100)	5 (12.8)	1 (5.3)	5 (17.2)	1 (4.5)	0.183	0.6689
CP-933N	*espK*	89 (100)	34 (87.2)	7 (36.8)	29 (100.0)	7 (31.8)	13.289	0.0003
LEE	*eae*	89 (100)	34 (87.2)	7 (36.8)	29 (100.0)	5 (22.7)	13.289	0.0003
LEE	*eae-gamma*	89 (100)	4 (10.3)	1 (5.3)	5 (17.2)	0 (0.0)	0.019	0.8906
LEE	*espF1*	89 (100)	39 (100)	14 (73.7)	29 (100.0)	17 (77.3)	8.139	0.0043
LEE	*espG*	89 (100)	35 (89.7)	7 (36.8)	29 (100.0)	6 (27.3)	15.348	<0.0001
OI-1	*espX1*	89 (100)	39 (100)	15 (78.9)	29 (100.0)	18 (81.8)	5.845	0.0156
OI-36	*nleD-2*	89 (100)	4 (10.3)	0 (0)	0 (0.0)	1 (4.5)	0.800	0.3709
OI-36	*nleH1-1*	89 (100)	27 (69.2)	3 (15.8)	21 (72.4)	2 (9.1)	12.551	0.0004
OI-37	*espX2*	89 (100)	4 (10.3)	2 (10.5)	5 (17.2)	1 (4.5)	0.001	0.9747
OI-43 and OI-48	*iha*	89 (100)	26 (66.7)	12 (63.2)	21 (72.4)	13 (59.1)	0.070	0.7919
OI-43 and OI-48	*terE*	89 (100)	36 (92.3)	10 (52.6)	29 (100.0)	10 (45.5)	9.958	0.0016
OI-43 and OI-48	*ureD*	89 (100)	36 (92.3)	8 (42.1)	29 (100.0)	8 (36.4)	14.949	0.0001
OI-44	*espV*	89 (100)	31 (79.5)	6 (31.6)	28 (96.6)	5 (22.7)	10.706	0.0011
OI-50	*espN*	89 (100)	31 (79.5)	3 (15.8)	27 (93.1)	2 (9.1)	18.825	<0.0001
OI-50	*espO1-1*	89 (100)	11 (28.2)	5 (26.3)	11 (37.9)	4 (18.2)	0.023	0.8799
OI-50	*espX7*	89 (100)	31 (79.5)	3 (15.8)	27 (93.1)	2 (9.1)	18.825	<0.0001
OI-57	*ecs1763*	89 (100)	29 (74.4)	6 (31.6)	27 (93.1)	6 (27.3)	8.065	0.0045
OI-62	*espR1*	89 (100)	39 (100)	16 (84.2)	29 (100.0)	19 (86.4)	3.674	0.0553
OI-71	*ecs1822*	89 (100)	29 (74.4)	2 (10.5)	25 (86.2)	2 (9.1)	18.435	<0.0001
OI-71	*espM1*	89 (100)	29 (74.4)	2 (10.5)	25 (86.2)	2 (9.1)	18.435	<0.0001
OI-71	*nleA*	88 (98.9)	25 (64.1)	6 (31.6)	21 (72.4)	6 (27.3)	4.203	0.0404
OI-71	*nleF*	88 (98.9)	33 (84.6)	5 (26.3)	25 (86.2)	6 (27.3)	16.727	<0.0001
OI-71	*nleG*	89 (100)	29 (74.4)	2 (10.5)	25 (86.2)	2 (9.1)	18.435	<0.0001
OI-71	*nleH1-2*	89 (100)	36 (92.3)	7 (36.8)	29 (100.0)	7 (31.8)	17.708	<0.0001
OI-79	*espJ*	89 (100)	36 (92.3)	5 (26.3)	29 (100.0)	5 (22.7)	23.763	<0.0001
OI-108	*espM2*	89 (100)	32 (82.1)	2 (10.5)	25 (86.2)	2 (9.1)	24.077	<0.0001
OI-108	*espW*	89 (100)	33 (84.6)	2 (10.5)	25 (86.2)	3 (13.6)	26.292	<0.0001
OI-122	*efa1*	89 (100)	32 (82.1)	3 (15.8)	29 (100.0)	1 (4.5)	20.754	<0.0001
OI-122	*efa2*	89 (100)	31 (79.5)	3 (15.8)	29 (100.0)	0 (0.0)	18.825	<0.0001
OI-122	*ent*	89 (100)	32 (82.1)	5 (26.3)	27 (93.1)	3 (13.6)	14.854	0.0001
OI-122	*nleB*	89 (100)	34 (87.2)	7 (36.8)	29 (100.0)	5 (22.7)	13.289	0.0003
OI-122	*nleE*	89 (100)	34 (87.2)	7 (36.8)	29 (100.0)	5 (22.7)	13.289	0.0003
OI-122	*pagC*	89 (100)	19 (48.7)	8 (42.1)	14 (48.3)	9 (40.9)	0.037	0.8466
OI-141 and OI-154	*lpfA*	89 (100)	5 (12.8)	2 (10.5)	5 (17.2)	2 (9.1)	0.063	0.8013
OI-153	*espY4-2*	89 (100)	1 (2.6)	0 (0)	0 (0.0)	1 (4.5)	0.496	0.4814
OI-174	*espX6*	89 (100)	1 (2.6)	0 (0)	1 (3.4)	0 (0.0)	0.496	0.4814
pO157	*ehxA*	89 (100)	36 (92.3)	15 (78.9)	29 (100.0)	15 (68.2)	1.074	0.3000
pO157	*espP*	86 (96.6)	29 (74.4)	14 (73.7)	23 (79.3)	14 (63.6)	0.003	0.9561
pO157	*etpD*	89 (100)	4 (10.3)	0 (0)	3 (10.3)	1 (4.5)	0.800	0.3709
pO157	*katP*	89 (100)	23 (59)	4 (21.1)	19 (65.5)	4 (18.2)	5.939	0.0148
pO157	*toxB*	89 (100)	21 (53.8)	3 (15.8)	20 (69.0)	3 (13.6)	6.140	0.0132

[a] C-I, *E. coli* island; LEE, locus for enterocyte effacement; OI, O island.
[b] χ^2 calculations are based on *E. coli* non-O157 from human and cattle data.

No significant differences were observed in the molecular profiles of O157:H7 strains linked to HUS and non-HUS cases, and the inclusion of additional genetic markers is needed for discrimination. Greater variations were seen in the molecular profiles of the non-O157 isolates, most likely due to the diversity of their serotypes. Many of the genetic markers tested were absent in the non-O157 strains, particularly those isolated from cattle. Almost all of the *nle* genes tested were present in the clinical O157:H7 and non-O157 strains, suggesting that they play an important role in virulence. The products of *nleB*, *nleD*, *nleE*, and *nleH* have been shown to inhibit host inflammatory responses (23), leading to more severe disease outcomes, and these are largely absent in our cattle strains. A similar distribution pattern was seen for most *esp* genes. As expected, isolates belonging to the most virulent seropathotypes had the highest proportion of virulence genes. Strains belonging to seropathotype A contained almost all of the virulence genes tested (stx_2, *espY1*, *espY3*, *eae-gamma*, *nleD-2*, *espX2*, *espO1-1*, *lpfA*, *espY4-2*, *espX6*, and *etpD*). The presence of genes *espK*, *eae*, *espG*, *terE*, *ureD*, *espV*, *espN*, *espX7*, *ecs1763*, *ecs1822*, *espM1*, *nleF*, *nleG*, *nleH1-2*, *espJ*, *espM2*, *espW*, *efa1*, *efa2*, *ent*, *nleB*,

TABLE 3 Shiga toxin subtyping of *E. coli* O157:H7 and non-O157 isolates

Shiga toxin	Prevalence (no. [%]) of:		
	O157:H7 ($n = 89$)	Clinical non-O157 ($n = 39$)	Cattle non-O157 ($n = 19$)
Shiga toxin gene			
stx_1	67 (72.8)	31 (79.5)	16 (80.0)
stx_2	89 (100.0)	14 (35.9)	7 (36.8)
Shiga toxin gene subtype			
stx_{1a}	0 (0)	22 (56.4)	12 (63.2)
stx_{1a}, stx_{2a}	67 (75.3)	5 (12.8)	1 (5.3)
stx_{1a}, stx_{2a}, stx_{2d}	0 (0)	0 (0)	1 (5.3)
stx_{1a}, stx_{2d}	0 (0)	0 (0)	2 (10.5)
stx_{1c}	0 (0)	1 (2.6)	0 (0)
stx_1 NT	0 (0)	2 (5.1)	0 (0)
stx_1 NT, stx_{2a}	0 (0)	1 (2.6)	0 (0)
stx_{2a}	19 (21.3)	5 (12.8)	1 (5.3)
stx_{2a}, stx_{2c}	3 (3.4)	0 (0)	0 (0)
stx_{2c}	0 (0)	2 (5.1)	0 (0)
stx_{2d}	0 (0)	1 (2.6)	0 (0)
stx_2 NT	0 (0)	0 (0)	2 (10.5)

and *nleE* can be used to differentiate strains belonging to seropathotypes B from those belonging to seropathotypes D and E. These data corroborate a recent study showing that these potential virulence genes were significantly more frequent among HUS-associated than non-HUS-associated strains (24).

Interestingly, five clinical non-O157 isolates lacking the *eae* gene were associated with human disease; four isolates also lacked stx_2 and three lacked most of the virulence genes tested. Genes *espF1*, *espR1*, and *espX1* were the only genes present in all of the O157 and non-O157 clinical strains tested. Further studies are required to address the role of these genes as related to human disease.

Our data support that HUS development is dependent on age-associated factors as shown in previous studies (25). Our findings correlate with those of a recent study showing that the age of the patient (<5 years) and the presence of *eae* and stx_{2a} genes in STEC showed significant associations with the development of HUS ($P < 0.05$ for each parameter), while stx_1-positive STEC was associated with non-HUS cases ($P < 0.05$) (24). Nonetheless, age is not the sole determining factor for disease progression, and additional investigation is needed to identify the genetic determinants of HUS. In conclusion, the panel of genes examined was present in almost all O157 STEC, but in non-O157 STEC, the presence/absence of these genes varies, even within a given serotype. Lastly, based on the gene profile results in this study we were not able to identify one or a combination of genetic markers that can reliably predict disease outcomes. Further studies using whole-genome sequencing might identify additional virulence markers and increase our understanding of their contribution to human disease.

TABLE 4 Patient demographics for *E. coli* O157:H7 and non-O157 cases

Characteristic	No. (%) of cases with:			
	O157:H7 from 2004–2012 ($n = 1,407$)	O157:H7 non-HUS subset ($n = 60$)	O157:H7 HUS subset ($n = 29$)	Clinical non-O157 ($n = 39$)[a]
Age (yr)				
<5	270 (19.2)	8 (13.3)	14 (48.3)	12 (30.8)
5–9	170 (12.1)	6 (10.0)	9 (31.0)	1 (2.6)
10–19	253 (18.0)	7 (11.7)	4 (13.8)	7 (17.9)
20–29	251 (17.8)	15 (25.0)	2 (6.9)	8 (20.5)
30–39	94 (6.7)	6 (10.0)	0 (0.0)	2 (5.1)
40–49	97 (6.9)	5 (8.3)	0 (0.0)	3 (7.7)
50–59	102 (7.2)	4 (6.7)	0 (0.0)	4 (10.3)
60–69	71 (5.0)	6 (10.0)	0 (0.0)	0 (0.0)
≥70	99 (7.0)	3 (5.0)	0 (0.0)	2 (5.1)
Mean	26.0	30.3	8.7	22.0
Gender				
Male	720 (51.2)	36 (60.0)	17 (58.6)	17 (43.6)
Female	687 (48.8)	24 (40.0)	12 (41.4)	22 (56.4)
Hospitalization				
Yes	345 (24.5)	23 (38.3)	29 (100)	3 (7.7)
No	925 (65.7)	33 (55.0)	0 (0.0)	36 (92.3)
Unknown	137 (9.7)	4 (6.7)	0 (0.0)	0 (0.0)

[a] HUS was not associated with any of the non-O157 isolates.

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